ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2001, p. 1939–1940 Vol. 45, No. 6
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.6.1939–1940.2001

Letter to the Editor

Carbapenem-Resistant *Klebsiella pneumoniae* in Singapore Producing IMP-1 β-Lactamase and Lacking an Outer Membrane Protein

IMP metallo-β-lactamases hydrolyze virtually all β-lactams and are insensitive to clinically available inhibitors. IMP-1 has been reported repeatedly since 1991 in Japan (8), where it is now scattered in *Pseudomonas aeruginosa* and *Serratia marcescens* and has been found in *Klebsiella pneumoniae* (H. Kurkawa, Y. Tetsuya, N. Shibata, K. Shibayama, and Y. Arakawa, Letter, Lancet 354:955, 1999). Carriage of bla_{IMP-1} has not been confirmed outside Japan, but related enzymes—IMP-2 and -4—have been found in acinetobacters in Italy (9) and Hong Kong (1); IMP-4 was also found in *Citrobacter youngae* in Guangzhou, China (2).

In 1999, we reported a carbapenem-resistant *K. pneumoniae* isolate (DB96) from blood cultures of a leukemic patient at Singapore General Hospital (T. H. Koh, G. S. Babini, N. Woodford, L.-H. Sng, L. M. C. Hall, and D. M. Livermore, Letter, Lancet 353:2162, 1999). The isolate had a pI 9.0 carbapenemase and gave a PCR product with primers to bla_{IMP}. Carbapenemase production was conjugatively transmissible to *Escherichia coli* in association with a 150-kb plasmid. We now report the sequence for the carbapenemase gene and show that other factors codetermined imipenem resistance.

When isolate DB96 was examined with E-tests (AB Biodisk, Solna, Sweden), confluent growth occurred up to an imipenem concentration of 3 μg/ml, but isolated colonies grew to the maximum drug concentration on the strip (32 μg/ml). One highly resistant colony, designated DB96M, was retained and was homogeneously resistant on retesting. Another variant, DB96R, was obtained after repeated subculture and showed no growth at imipenem concentrations above 3 μg/ml. MICs were determined by NCCLS broth microdilution (7). Organisms DB96 and DB96M had identical resistance levels (±1 dilution) (Table 1) and had high-level resistance to all β-lactams including carbapenems; DB96R was less resistant only to carbapenems. All three variants retained the pI 9.0 carbapenemase, as detected by isoelectric focusing. Imipenemase specific activities were 0.69, 0.75, and 0.87 μmol of imipenem/min/mg of protein for DB96, DB96M, and DB96R, respectively, as determined by the method of Livermore and Williams (6); these values were not significantly different.

TABLE 1. MICs for *K. pneumoniae* isolates, as determined by NCCLS broth dilution

Antibiotic	MIC (μg/ml)		
	DB96[a]	DB96M[b]	DB96R[c]
Imipenem	>128	>128	4
Meropenem	128	128	8
Ceftazidime	>128	>128	>128
Ceftazidime-clavulanate (4 μg/ml)	>128	>128	>128
Cefotaxime	>128	>128	>128
Cefuroxime	>128	>128	>128
Cefepime	>128	>128	64
Cefoxitin	>128	>128	>128
Cefoxitin-cloxacillin (100 μg/ml)	>128	>128	>128
Cefotetan	>128	>128	>128
Piperacillin	>128	>128	>128
Piperacillin-tazobactam (4 μg/ml)	>128	>128	>128
Aztreonam	>128	>128	>128
Amikacin	1	1	0.5
Ciprofloxacin	8	8	8
Gentamicin	>128	>128	>128
Chloramphenicol	>128	>128	>128
Trimethoprim	>128	>128	>128

[a] Isolate.
[b] Selected as growing at an imipenem concentration of 32 μg/ml on an E-test strip.
[c] Selected, after repeated subculture, as not yielding highly carbapenem-resistant variants such as DB96M.

Using primers based on published sequences for *P. aeruginosa* 101/1477 (5), two amplicons were generated from DB96. First, primers bla_{IMP-1} 1up (5′-GTGGGTCGATGTTTGATG TTAT-3′; positions 1400 to 1421) and bla_{IMP-1} 6dn (5′-TGCG CGTTGTGGAATACTTTGC-3′; positions 2298 to 2319), which flank the open reading frame by ca. 60 bp upstream and 70 bp downstream, respectively, were used to generate an amplicon of approximately 1 kb. Secondly, primers bla_{IMP-1} 2up (5′-CTTGATGAAGGCGTTTATGTT-3′; positions 1572 to 1592) and bla_{IMP-1} 5dn (5′-TAACCGCCTGCTCTAATGT AAG-3′; positions 2160 to 2181), which were ca. 90 bp and 70 bp internal to the start and stop codons, respectively, were used to generate an amplicon of ca. 600 bp internal to the 1-kb amplicon. Both amplicons were then sequenced using primers bla_{IMP-1} 3up (5′-ACGGTAAGGTTCAAGCCACAA-3′; positions 1864 to 1884) and bla_{IMP-1} 4dn (5′-TTTCAGGCAACC AAACCACTA-3′; positions 1969 to 1989), which were central to the open reading frame and original primers. The aligned sequence was submitted to BLAST 2.0 and found to be identical to bla_{IMP-1} from *S. marcescens* (GenBank accession number S71932) (8), *P. aeruginosa* (AJ223604) (5), and *K. pneumoniae* (D29636). This is the first confirmation of a classical bla_{IMP-1} outside Japan. The patient had no history of recent travel to Japan. Undetected importation by other patients or travellers is possible; alternatively, bla_{IMP-1} may have escaped to plasmids independently in Singapore.

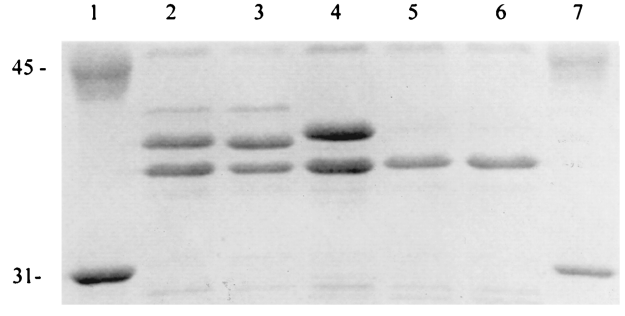

FIG. 1. Outer membrane profiles of *K. pneumoniae* isolates in SDS-PAGE. Lanes 1 and 7, molecular weight markers (in kilodaltons); lane 2, carbapenem-susceptible control isolate 207; lane 3, carbapenem-susceptible control isolate 504; lane 4, DB96R; lane 5, DB96; lane 6, DB96M. (The photograph has been cropped, so not all molecular weight markers can be seen.)

Outer membrane proteins (OMPs) were extracted (6) and electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4). Organisms DB96 and DB96M showed greatly diminished expression of a major 39-kDa OMP compared with DB96R and with carbapenem-susceptible *Klebsiella* controls (Fig. 1). DB96, DB96M, and DB96R all lacked the minor 41-kDa OMP present in the controls. It seems likely, from its mass, that the 39-kDa OMP corresponds to a major porin (3) and that high-level resistance to carbapenems demands impermeability as well as an IMP β-lactamase. This conclusion is supported by the low imipenem MICs (2 μg/ml) for IMP-1-positive *E. coli* transconjugants of strain DB96 (Koh et al., letter, 1999). Because IMP-1 alone does not confer high-level carbapenem resistance in *Enterobacteriaceae*, it might spread without attracting attention, and microbiologists should be aware that gram-negative bacteria with borderline susceptibility to carbapenems could be IMP producers. Suspicious isolates should have carbapenem MICs checked and be examined for carbapenemase activity.

This work was supported by an MRCPath project grant from the British Society for Antimicrobial Chemotherapy.

We are grateful to Brigid Duke, Jeff Maskell, Mei Yuan, and David Griffiths for advice and assistance.

REFERENCES

1. **Chu, Y.-W., M. Afzal-Shah, E. T. S. Houang, M.-F. Palepou, D. J. Lyon, N. Woodford, and D. M. Livermore.** 2001. IMP-4, a novel metallo-β-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. Antimicrob. Agents Chemother. **45:**710–714.
2. **Hawkey, P. M., J. Xiong, H. Ye, H. Li, and F. H. M'Zali.** 2001. Occurrence of a new metallo-beta-lactamase IMP-4 carried on a conjugative plasmid in *Citrobacter youngae* from the People's Republic of China. FEMS Microbiol. Lett. **194:**53–57.
3. **Hernandez-Alles, S., S. Alberti, D. Alvarez, A. Domenech-Sanchez, L. Martinez-Martinez, J. Gil, J. M. Tomas, and V. J. Benedi.** 1999. Porin expression in clinical isolates of *Klebsiella pneumoniae*. Microbiology **145:**673–679.
4. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **277:**680–685.
5. **Laraki, N., M. Galleni, I. Thamm, M. L. Riccio, G. Amicosante, J.-M. Frère, and G. M. Rossolini.** 1999. Structure of In31, a bla_{IMP}-containing *Pseudomonas aeruginosa* integron phyletically related to In5, which carries an unusual array of gene cassettes. Antimicrob. Agents Chemother. **43:**890–901.
6. **Livermore, D. M., and J. D. Williams.** 1996. β-Lactams: mode of action and mechanisms of bacterial resistance, p. 502–578. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 4th ed. Williams & Wilkins, Baltimore, Md.
7. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7–A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. **Osano, E., Y. Arakawa, R. Wacharotayankun, M. Ohta, T. Horii, H. Ito, F. Yoshimura, and N. Kato.** 1994. Molecular characterization of an enterobacterial metallo β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob. Agents Chemother. **38:**71–78.
9. **Riccio, M. L., N. Franceschini, L. Boschi, B. Caravelli, G. Cornaglia, R. Fontana, G. Amicosante, and G. M. Rossolini.** 2000. Characterization of the metallo-β-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla_{IMP} alleleic variants carried by gene cassettes of different phylogeny. Antimicrob. Agents Chemother. **44:**1229–1235.

Tse H. Koh*
Li-Hwei Sng
Singapore General Hospital
Singapore 169608, Singapore

Gioia S. Babini
Neil Woodford
David M. Livermore
Central Public Health Laboratory
London, United Kingdom

Lucinda M. C. Hall
St. Bartholomew's and the Royal London School of Medicine and Dentistry
London, United Kingdom

*Phone: 65-321-4505
Fax: 65-222-6826
E-mail: gptthk@sgh.com.sg